

# Ethylene Effects

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**Introduction:** Ethylene ( $C_2H_4$ ) is a simple naturally occurring organic molecule that is a colorless gas at biological temperatures.

## *Biological Attributes of Ethylene:*

Colorless gas at biological temperatures.

Naturally occurring organic compound.

Readily diffuses from tissue.

Produced from methionine via ACC by a highly regulated metabolic pathway.

Key enzymes are ACC synthase and ACC oxidase.

$C_2H_4$  synthesis is inhibited by  $C_2H_4$  in vegetative and immature reproductive tissue.

$C_2H_4$  synthesis is promoted (autocatalytic) by  $C_2H_4$  in mature reproductive climacteric tissue.

Effective at ppm and ppb concentrations ( $1 \text{ ppm} = 6.5 \times 10^{-9} \text{ M}$  at  $25^\circ\text{C}$ ).

Requires  $O_2$  to be synthesized, and  $O_2$  and low levels of  $CO_2$  to be active.

Many biotic and abiotic sources contribute to the presence of  $C_2H_4$  in the postharvest environment. Ripening and diseased plant tissues are a significant source of  $C_2H_4$ , as are industrial sources, the most prominent ones being internal combustion engines and fires.

Ethylene is biologically active at very low concentration measured in the ppm and ppb range. Most plants synthesize small amounts of  $C_2H_4$  that appear to coordinate growth and development. Because it is a gas,  $C_2H_4$  readily diffuses from sites of production, and continuous synthesis is needed to maintain biologically active levels in the tissues. Barriers to diffusive loss not only include the commodity's epidermis, but also postharvest coatings and packaging. Under biotic or abiotic stress, or during climacteric ripening,  $C_2H_4$  production can increase dramatically, and emanations from stimulated tissue can accumulate in packages or storerooms and produce unwanted effects in adjacent tissue. Other molecules with specific configurations can mimic  $C_2H_4$ , but are less effective. For example,  $C_2H_4$  analogs propylene ( $C_3H_6$ ) and acetylene ( $C_2H_2$ ) require 100- and 2,700-fold, respectively, the concentration of  $C_2H_4$  to elicit the same effect.

Plants produce  $C_2H_4$  through an actively regulated biosynthetic pathway in which the amino acid methionine is converted to ACC (1-aminocyclopropane-1-carboxylic acid) and then to  $C_2H_4$  through a series of biochemical reactions.  $O_2$  is required for the synthesis of  $C_2H_4$  and both  $O_2$  and  $CO_2$  are required for its biological activity. Each reaction in the synthesis and action of  $C_2H_4$  involves a biological catalyst, i.e., an enzyme, that focuses the reaction into producing the next specific chemical for that pathway. Enzyme activity is regulated either through its synthesis and/or destruction, or by interactions with substrates and products. These interactions can create a positive or a negative feedback of  $C_2H_4$  on its synthesis (Fig. 1).

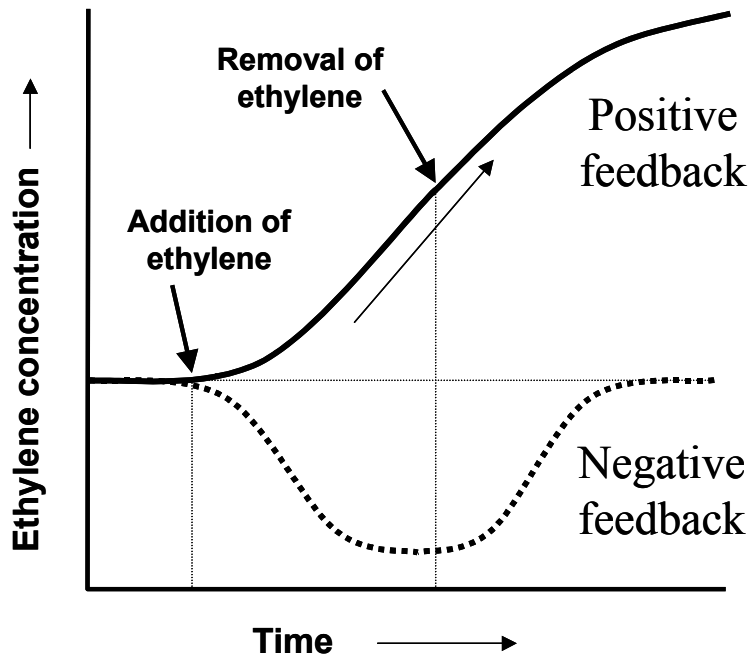


Figure 1. Effect of adding and removing ethylene from the atmosphere surrounding tissues that respond with a positive (ethylene promotes its own synthesis) or negative (ethylene inhibits its own synthesis) feedback (modified from Saltveit, 1999).

In vegetative tissue and in non-climacteric and immature climacteric fruit tissue  $C_2H_4$  suppresses its own synthesis and in ripening climacteric fruit  $C_2H_4$  enhances its own synthesis. This positive feedback of  $C_2H_4$  on  $C_2H_4$  synthesis is called autocatalytic  $C_2H_4$  production.

Plants respond to  $C_2H_4$  in a number of ways:

*Ethylene stimulates:*

- Synthesis of  $C_2H_4$  in ripening climacteric fruit
- Ripening of climacteric fruit and some non-climacteric fruit.
- Anthocyanin synthesis in ripening fruit.
- Chlorophyll destruction and yellowing (eg., degreening of citrus).
- Seed germination.
- Adventitious root formation.
- Respiration & phenylpropanoid metabolism.
- Flower initiation in bromeliads, eg., pineapple.
- Abscission and senescence.

*Ethylene inhibits:*

- Ethylene synthesis in vegetative tissue and non-climacteric fruit.
- Flowering and flower development in most plants.
- Auxin transport.
- Shoot and root elongation, ie., growth.

Depending on a number of variables,  $C_2H_4$  has both beneficial and deleterious effects on harvested fruits, vegetables, and ornamentals:

*Beneficial effects:*

Promotes color development in fruit.  
Stimulates ripening of climacteric fruit.  
Promotes de-greening of citrus.  
Stimulates dehiscence in nuts.  
Alters sex expression (cucurbitaceae).  
Promotes flowering in (eg., pineapple).  
Reduces lodging of cereals.

*Detrimental effects:*

Accelerates senescence.  
Enhances excessive softening of fruits.  
Stimulates chlorophyll loss (eg., yellowing).  
Stimulates sprouting of potato.  
Promotes discoloration (eg., browning).  
Promotes abscission of leaves and flowers.  
Stimulates phenylpropanoid metabolism.

Often an  $C_2H_4$ -induced change in one commodity is viewed as beneficial, while the same change in another commodity is viewed as detrimental. For example,  $C_2H_4$  is used to promote: ripening of bananas, melons, and tomatoes; degreening of oranges; and synthesis of pigments in apples. Yet the same changes are unwanted when  $C_2H_4$  promotes: over-ripening of fruit; yellowing of broccoli; development of brown russet spot lesions in lettuce; and senescence of flowers. Because of these diverse and often opposite effects of  $C_2H_4$ , controlling its action in plants is of great economic importance to producers, wholesalers, retailers and consumers of fresh fruits, vegetables, and ornamentals.

In most vegetative tissues,  $C_2H_4$  is only produced in biologically active amounts during early stages of development, or in response to a biotic or abiotic stress. Mutant plants that do not respond to  $C_2H_4$  often grow normally, with only a few insignificant alterations in development. Most of the effects of  $C_2H_4$  on vegetative tissue are therefore the result of the tissue's response to a stress or to the intentional or unintentional exposure of tissue to active levels of  $C_2H_4$ .

In contrast to vegetative tissue, biologically produced  $C_2H_4$  plays a crucial role in the development of reproductive tissues and in the ripening of certain climacteric fruit. The rates of  $C_2H_4$  production and its internal concentration often vary by orders of magnitude during early stages of development and during the initiation and development of reproductive structures. Increased rates of  $C_2H_4$  production are especially pronounced during the ripening of climacteric fruit such as apples, avocados, bananas, melons, pears, and tomatoes. In these fruit, the autocatalytic production of  $C_2H_4$  heralds the onset of ripening and is required for many of the reactions associated with ripening to continue. The ethylene production rates of many fruits and vegetables is summarized in Table 3 of the "General Introduction" section.

Once internal  $C_2H_4$  exceeds a level characteristic for the species, tissue and developmental stage, the further production of  $C_2H_4$  is stimulated by presence of previously produced  $C_2H_4$ . In this way, autocatalytic positive feedback can increase rates of  $C_2H_4$  production and internal concentration of  $C_2H_4$  by a 1000-fold during ripening. External application of  $C_2H_4$  can promote the ripening of climacteric fruit, eg., avocado, banana, honeydew, and tomato, and beneficial quality changes in non-climacteric fruit, eg., degreening of lemon and orange. Once autocatalytic  $C_2H_4$  production has started in climacteric fruit, lowering its external concentration has an insignificant effect on its internal levels, rates of production, or action.

Ethylene is an important plant growth regulator that has pronounced effects on many aspects of plant growth and development. Regulating its effectiveness is commercially important for many crops. Controlling its effectiveness can mean either increasing its beneficial effects or decreasing its detrimental effects. There are a number of ways to accomplish either objective.

*Reducing Effectiveness of Ethylene:*

Use ethylene tolerant cultivars.  
Keep atmosphere free of  $C_2H_4$ .  
Maintain at coldest possible temperature.  
Store under CA or MA, or in MAP.  
Minimize time between exposure and use.

*Increasing Effectiveness of Ethylene:*

Use  $C_2H_4$  sensitive cultivars.  
Keep an active level of  $C_2H_4$  in the air.  
Maintain at optimum temperature.  
Store under adequate levels of  $O_2$  and  $CO_2$ .  
Allow sufficient time for plant response.

**Ethylene Interactions in Plants:** There are some significant interactions between the plant and its environment that are important in understanding how to control biological activity of  $C_2H_4$  in plants (Fig. 2).

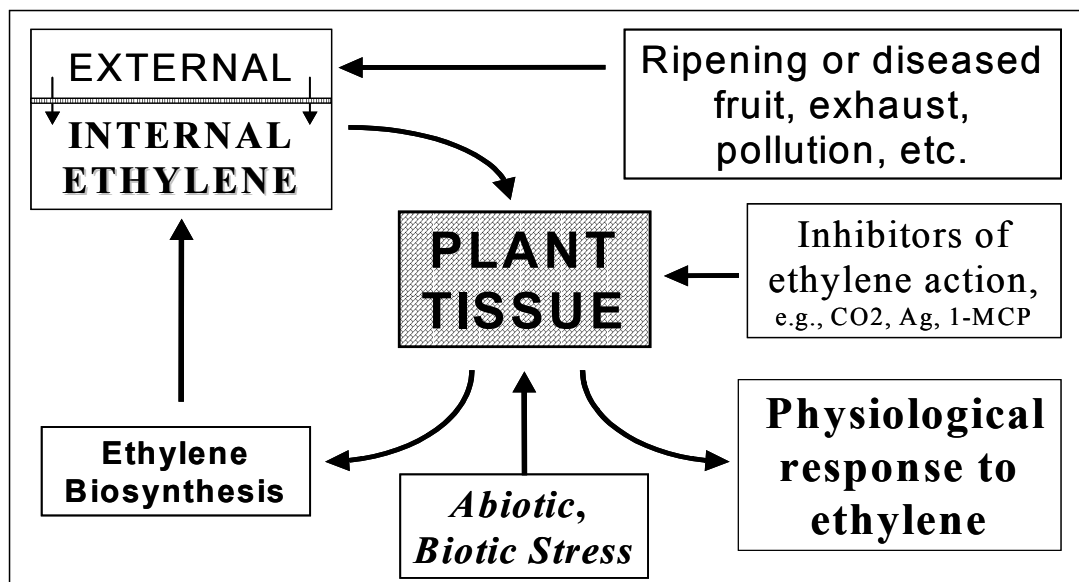


Fig. 2. Interactions among the plant and ethylene in its environment (Saltveit, 1999).

Ethylene in the atmosphere can have a direct effect on plant tissue by raising the internal concentration to an active level. Sources of atmospheric  $C_2H_4$  include exhaust from trucks and forklifts, pollution from industrial activity and the burning of fuels, and biosynthesis by diseased plants or ripening fruit. In some cases  $C_2H_4$  - whether applied as a gas or as a  $C_2H_4$  releasing compound such as ethephon - is intentionally added to the plant's environment to stimulate desirable changes. The changes can include promotion of flowering in pineapple, ripening of avocado, banana, melon, and tomato fruit, degreening of citrus, altering sex expression in cucurbits, defoliation, promotion of latex secretion, and many others.

The activity of  $C_2H_4$  inside plants is not only regulated by the absolute level of  $C_2H_4$ , but also by the responsiveness of tissues and the presence of  $CO_2$ , the natural antagonist of  $C_2H_4$  action. The response of plants to  $C_2H_4$ , therefore, depends on a number of factors, only one of which is the rate of  $C_2H_4$  production by the plant. Tissue sensitivity is dependent on species, cultivar, cultural practices, and stage of development.

Prior and current stresses have a significant effect on modulating the effect of  $C_2H_4$ . For example, wounding stimulates both  $C_2H_4$  production and a host of plant defense responses such as increased phenylpropanoid metabolism. Some of these responses involve  $C_2H_4$ , and others do not. Increased phenolic metabolism greatly increases the susceptibility of some crops like lettuce to develop browning, eg., russet spotting, when exposed to  $C_2H_4$  and/or mechanical injury.

The effect of tissue susceptibility is most clearly seen in fruit tissue. Immature climacteric fruit respond to  $C_2H_4$  with increased respiration and reduced  $C_2H_4$  production. Once the tissue has reached a certain stage of maturity, however,  $C_2H_4$  not only promotes increased respiration, but also increased  $C_2H_4$  synthesis.

Controlling the effectiveness of  $C_2H_4$  does not always involve a reduction in its activity. There are many beneficial effects of  $C_2H_4$  that can be enhanced (see above). The techniques used to increase the effectiveness of  $C_2H_4$  are almost the mirror image of techniques used to reduce its effectiveness.

Ethylene action can be enhanced by using cultivars that are sensitive and respond uniformly to  $C_2H_4$  rather than cultivars that are  $C_2H_4$  insensitive. An effective concentration of  $C_2H_4$  should be maintained

around the tissue for a sufficient time to elicit the full response. However, since the response to  $C_2H_4$  is log-linear (a log increase in  $C_2H_4$  concentration results in a linear increase in the response), there is an extremely large range over which the concentrations are effective. The application of  $C_2H_4$  must be at the proper stage of development and at the proper temperature for the desired effects to be induced. Ethephon, and similar  $C_2H_4$  releasing chemicals, permit the commercial application of  $C_2H_4$  in the field. After harvest,  $C_2H_4$  gas, either from compressed gas cylinders or catalytically generated from alcohol can be used in enclosed storage rooms.

**Controlling Ethylene Action:** There are roughly three ways to control the action of  $C_2H_4$  in plants. The first is to prevent the plant from being exposed to biologically active levels of  $C_2H_4$ . The second is to prevent the plant tissue from perceiving the  $C_2H_4$  that is in its surrounding atmosphere or that is being produced by the tissue. The third is to prevent the plant from responding to the perceived  $C_2H_4$  by controlling exposure to  $C_2H_4$ . It is important to:

- Keep the air around the commodity  $C_2H_4$  free.
- Use fresh,  $C_2H_4$ -free air from outside.
- Scrub  $C_2H_4$  from the storage atmosphere.
- Use sachets of  $C_2H_4$  absorbers inside packages to reduce levels.
- Segregate  $C_2H_4$  producing commodities from sensitive ones.
- Keep exposure to a minimum (duration, level).
- Inhibit  $C_2H_4$  synthesis (AVG, ACC synthase; Low  $O_2$ , ACC oxidase)

This is not much of a problem in the field, since the levels of  $C_2H_4$  found even in polluted air rarely reach biologically active levels. However, in greenhouses, cold-storage-rooms, and transportation vehicles  $C_2H_4$  can frequently accumulate to biologically active levels. Ethylene found in these enclosed spaces comes from varied sources, but the two most prominent are from diseased, stressed or ripening plant tissue, and from the incomplete combustion of organic fuels.

With proper ventilation of enclosed spaces, and persistent attention to the condition of adjacent plants and the operation of heaters and gas-powered fork-lifts,  $C_2H_4$  can be kept below biologically active levels. Sometimes, the  $C_2H_4$  that we are concerned with comes from the plant itself. Application of inhibitors of  $C_2H_4$  biosynthesis, eg., AVG and AOA, to the tissue before or after harvest can significantly reduce this source of  $C_2H_4$  exposure. For example, tissue can be prevented from making either stress or autocatalytic  $C_2H_4$  by blocking the biosynthetic pathway for  $C_2H_4$  synthesis. If exposure cannot be prevented, or has already occurred, then the duration of exposure and the level of  $C_2H_4$  in the atmosphere should both be kept as low as possible.

*Prevent Perception of Ethylene:* If significant amounts of  $C_2H_4$  are in the immediate environment, certain methods can be used to block the perception of  $C_2H_4$  by the plant:

- Store at coldest possible temperature.
- Use inhibitor of  $C_2H_4$  perception.
  - $CO_2$
  - Silver (eg., silver thiosulfate)
  - 1-Methyl cyclopropene (1-MCP)
- Use  $C_2H_4$ -insensitive cultivars.
- Interrupt the  $C_2H_4$ -induced signal.

Since perception is a metabolic process, holding the tissue at the lowest possible temperature will effectively reduce perception. Specific chemical inhibitors can also be used that directly interfere with the perception event.

A gaseous inhibitor like CO<sub>2</sub> or 1-MCP (1-methylcyclopropene) can be introduced into the atmosphere. The tissue can be dipped or fed a nonvolatile inhibitor like silver; but this treatment is limited to non-food crops. Ethylene resistant cultivars can be selected or the tissue can be genetically engineered to lack the necessary biochemical receptors for ethylene or the signal pathway necessary to transduce the signal into a physiological event.

Even after the molecular perception event has occurred, blocking the transduced signal will effectively prevent perception. However, effective methods to do this will require a far greater understanding of the signal pathway than is currently available.

*Prevent Response by the Plant:* The third way to control C<sub>2</sub>H<sub>4</sub> is to prevent the plant from responding to the perceived C<sub>2</sub>H<sub>4</sub>. This can be done by interfering with the metabolic machinery that is induced by exposure to C<sub>2</sub>H<sub>4</sub>:

- Store at coldest possible temperature.

- Store under CA or MA, or in MAP.

- Inhibit or reduce specific enzyme activity using chemical inhibitors (eg., AIP) or genetic engineering (eg., antisense or other gene knockout techniques).

- Divert protein synthesis, eg., heat-shock.

- Minimize time before use, eg., consumption.

Since all the effects of C<sub>2</sub>H<sub>4</sub> on plants that we are interested in involve metabolic changes, reducing the rate of metabolism by lowering the temperature, withholding a vital reactant (eg., O<sub>2</sub>), or by inhibiting a specific enzyme (eg., with a chemical or through genetic engineering) will prevent the response to C<sub>2</sub>H<sub>4</sub>.

For example, ripening promoted by C<sub>2</sub>H<sub>4</sub> often entails tissue softening that significantly reduces shelf-life. Using antisense technology to reduce the activity of enzymes involved in tissue softening has produced fruit that remain firmer longer. Ethylene also promotes phenylpropanoid metabolism in many tissues that use stress produced C<sub>2</sub>H<sub>4</sub> as a signal to induce defense mechanisms. Interfering with synthesis or activity of phenylalanine-ammonia lyase (PAL; first enzyme in phenolic metabolism) with chemical inhibitors or heat treatment eliminates tissue response to C<sub>2</sub>H<sub>4</sub>, preventing development of postharvest disorders.

**Application of Ethylene:** The quality of some fruit is increased when they are harvested at a mature stage that can withstand the rigors and duration of transport and then treated with C<sub>2</sub>H<sub>4</sub> to promote ripening before sale. These fruit include avocados, bananas, honeydew melons, lemons, oranges and tomatoes. An effective atmosphere of 100 to 150 μL L<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> in air can be produced by a number of methods. The 'shot' method introduces a relative large amount of gaseous C<sub>2</sub>H<sub>4</sub> into a ripening room by metering C<sub>2</sub>H<sub>4</sub> from compressed gas cylinders. Ethylene and air mixtures between 3.1% and 32% are explosive. While these concentrations are more than 200-fold higher than recommended, they have been reached when metering equipment has malfunctioned. Use of compressed gas containing around 3.1% C<sub>2</sub>H<sub>4</sub> in N<sub>2</sub>, ie., 'banana gas,' eliminates this problem. Catalytic converters are instruments that use a heated metal catalyst to convert alcohol into C<sub>2</sub>H<sub>4</sub>. They deliver a continuous flow of low C<sub>2</sub>H<sub>4</sub> into the storage room. Ethylene can also be applied in aqueous form from decomposition of compounds such as Ethrel. While stable at acidic pH, Ethrel quickly breaks down to C<sub>2</sub>H<sub>4</sub> as temperature and pH increase. Field application is approved for many food crops, but postharvest application is not approved.

Treatment with C<sub>2</sub>H<sub>4</sub> stimulates many metabolic pathways, including respiration. Oxygen use is increased, as is the production of CO<sub>2</sub> and heat. Rooms designed to hold produce being exposed to C<sub>2</sub>H<sub>4</sub> must be designed with extra air moving capacity to insure that an optimal ripening environment is maintained around the crop. Exposure to C<sub>2</sub>H<sub>4</sub> must be uniform through out the room and within packages. Heat of respiration and excessive CO<sub>2</sub> must be removed to maintain the proper environment. Loss of water by the crop will be increased by the rise in respiratory heat production. Maintaining a high RH can lessen water loss, but too much water vapor can decrease the strength of cardboard boxes and

promote pathogen growth. Judicious maintenance of proper ripening environments will insure production of high quality fruit. Care must be exercised in venting and opening ripening rooms to prevent release of sufficient amounts of C<sub>2</sub>H<sub>4</sub> to adversely affect other commodities stored in the same warehouse.

**Conclusion:** Ethylene can be both beneficial and detrimental to the storage of horticultural crops. Practical uses for C<sub>2</sub>H<sub>4</sub> and treatments to minimize its adverse effects have slowly accumulated over almost a century of study. The three general methods used to modulate C<sub>2</sub>H<sub>4</sub> activity involve controlling exposure, altering perception, and varying the response of the tissue. An understanding of ethylene's synthetic pathway and mode of action has greatly expanded the ability of Postharvest physiologists to devise treatments and storage conditions to control C<sub>2</sub>H<sub>4</sub> during the commercial storage and handling of horticultural crops. Simple methods like ventilation and temperature management can be combined with more sophisticated treatments like MAP and inhibitors of specific induced enzymes to provide conditions that optimize both storage-life and product quality.

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